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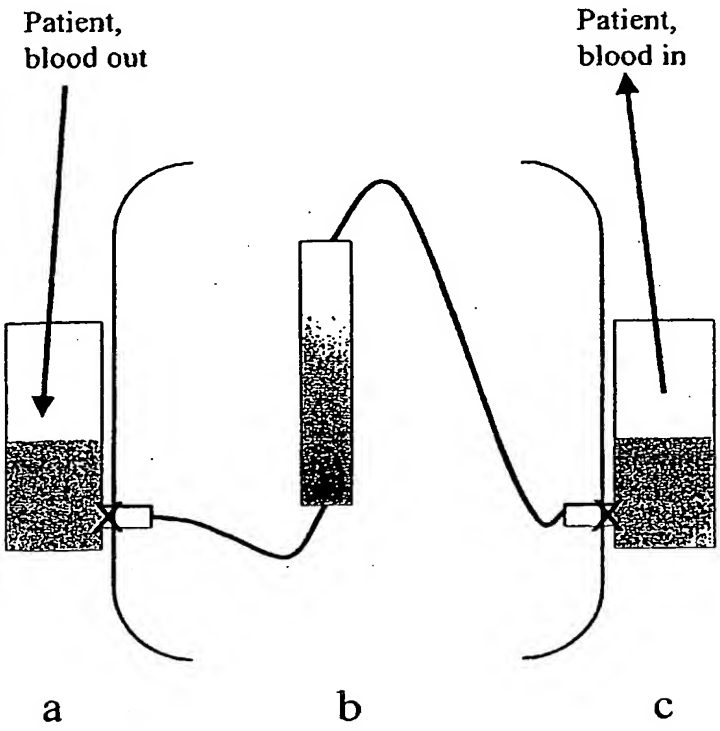
[Continued on next page]

(54) Title: AN EXTRACORPOREAL STABILISED EXPANDED BED ADSORPTION METHOD FOR THE TREATMENT OF SEPSIS



(57) Abstract: The present invention provides an extracorporeal adsorption method for removing harmful substances from blood in a way that is practicable in everyday clinical practice and applicable for the timely intervention to present the development of sepsis. Said extracorporeal adsorption method being effected by an adsorption column assembly where the adsorption column assembly comprising a column and an adsorption medium in the form of particles. The sedimented volume of said particles being at the most 80% of the volume of the column.

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Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
1 July 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

AMENDED CLAIMS

[received by the International Bureau on 07 July 2004 (07.07.04);
original claims 2, 13-26 have been replaced by amended claims 2, 13-26.
original claims 1, 3-12 remains unchanged

1. An extracorporeal adsorption method for removing harmful substances responsible of inducing sepsis caused by Gram-negative in a mammal, said extracorporeal adsorption method being effected by an adsorption column assembly, said adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of said particles being at the most 80% of the volume of the column, said particles being characterised by carrying an affinity specific molecule with a specific affinity for the LPS portion of said Gram-negative bacteria, said method comprising treating blood obtained from said mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the particles is formed.

2. An extracorporeal adsorption method for removing harmful substances responsible of inducing sepsis caused by Gram-negative or Gram-positive bacteria in a mammal, said extracorporeal adsorption method being effected by an adsorption column assembly, said adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of said particles being at the most 80% of the volume of the column, said particles being characterised by carrying an affinity specific molecule with a specific affinity for:

i) the LPS portion of said Gram-negative bacteria, and/or
ii) Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria,

said method comprising treating blood obtained from said mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the particles is formed.

3. A method according to claim 1 or 2 wherein the treated blood is capable of being reinfused into the same mammal.

4. A method according to any of claims 1-3, wherein the adsorption column assembly is adapted for fluidised bed adsorption, in particular stabilised fluidised bed adsorption.

5. A method according to any of the preceding claims, wherein the particles have a density of at least 1.3 g/ml and a mean diameter in the range of 5-1000 µm, such as a density of at least 1.5 g/ml and a mean diameter in the range of 5-300 µm, preferably a density of at least 1.8 g/ml and a mean diameter in the range of 5-150 µm, and most preferred a density of more than 2.5 g/ml and a mean diameter in the range of 5-75 µm.

6. A method according to any of the preceding claims, wherein the mammal is a human being.

7. A method according to any of the preceding claims, wherein the affinity specific molecule is selected from the group consisting of immunoglobulins, peptides, oligonucleotides, receptor proteins, antibiotics, and lectins.

8. A method according to any of the preceding claims, wherein two or more different affinity specific molecules are present on particles within the adsorption medium.

9. A method according to claim 6 or 7, wherein the affinity specific molecules are selected from immunoglobulins.

10. A method according to any of the preceding claims, wherein the affinity specific molecule is Polymyxin B.

11. A method according any of the preceding claims, wherein the affinity specific molecule is selected from the group consisting of a Toll-like receptor, most preferably TLR4 or binding fragments thereof or multimeric arrangements thereof, CD14, MD2, TLR2 and LBP, and any combination thereof.

12. A method according to any of the preceding claims, wherein the sedimented volume of the particles is at the most 70% of the volume of the column, such as at the most 60% of the volume of the column, e.g. at the most 50% of the volume of the column.

13. Use of an adsorption medium for the preparation of a therapeutic adsorption column assembly for the continuos therapeutic treatment of sepsis caused by Gram-negative bacteria in a mammal by extracorporeal adsorption, said adsorption column assembly comprising (i) a vessel for continuos obtaining blood from said mammal, (ii) a column comprising the adsorption medium, the sedimented volume of said adsorption medium being at the most 80% of the volume of the column, said adsorption medium being characterised by carrying an affinity specific molecule with a specific affinity for the LPS portion of said Gram-negative bacteria, said column is treating the obtained blood by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the adsorption medium is formed, and (iii) another vessel which continuously delivers blood back to the patient.

14. Use of an adsorption medium for the preparation of a therapeutic adsorption column assembly for the therapeutic treatment of sepsis caused by Gram-negative or Gram-postive bacteria in a mammal by extracorporeal adsorption, said adsorption column assembly comprising (a) a vessel for continuos obtaining blood from said mammal, (b) a column and the adsorption medium, the sedimented volume of said adsorption medium being at the most 80% of the volume of the column, said adsorption medium being characterised by carrying an affinity specific molecule with a specific affinity for:

i) the LPS portion of said Gram-negative bacteria, and/or

ii) Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria,

said column is treating the obtained blood by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the adsorption medium is formed, and

(c) another vessel which continuously delivers blood back to the patient.

15. The use according claim 13 or 14, wherein the flow rate of the blood through the column assembly is such that expansion ratio of the fluidised bed is at least 1.3, such as at least 1.5.

16. The use according to any of the claim 12-15, wherein the steps (a), (b) and (c) are preceded by a initial step by which a substance is first injected into the blood stream of the mammal.

17. The use according to any of the claims 13-16, wherein the mammal is a human being.

18. The use according to any of the claims 13-17, wherein the particles have a density of at least 1.3 g/ml and a mean diameter in the range of 5-1000 μm, such as a density of at least 1.5 g/ml and a mean diameter in the range of 5-300 μm, preferably a density of at least 1.8 g/ml and a mean diameter in the range of 5-150 μm, and most preferred a density of more than 2.5 g/ml and a mean diameter in the range of 5-75 μm.

19. A use according to any of claims 11-18, wherein the stabilised fluidised bed is placed in line with a switch capable of being activated when a blood substance reaches a pre-set value, said blood substance is monitored by a device, said device is placed in line with the blood circulation, said device sending the activating signal to the switch when said value is reached.

20. The use according to any of the claims 13-19, wherein the affinity specific molecule is selected from the group consisting of immunoglobulins, peptides, oligonucleotides, receptor proteins, antibiotics, and lectins.

21. The use according to any of the claims 13-20, wherein two or more different affinity specific molecules are present on particles within the adsorption medium.

22. The use according to claim 20 or 21, wherein the affinity specific molecules are selected from immunoglobulins.

23. The use according to any of the claims 20 or 21, wherein the affinity specific molecule is Polymyxin B.

24. A use according to claims 13-23, wherein the affinity specific molecule is selected from the group consisting of a Toll-like receptor, most preferably TLR4 or binding fragments thereof or multimeric arrangements thereof, CD14, MD2, TLR2 and LBP, and any combination thereof.

25. The use according to any of the claims 13-24, wherein the sedimented volume of the particles is at the most 70% of the volume of the column, such as at the most 60% of the volume of the column, e.g. at the most 50% of the volume of the column.

26. The use according to any of the claims 13-25, wherein the flow rate is such that stabilised fluidised bed of the particles is formed.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 03/00483

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01D15/00 G01N33/50 B01J8/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01D G01N B01J C07K A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 1 057 529 A (TORAY INDUSTRIES) 6 December 2000 (2000-12-06) abstract	4-26
A	page 2	1-3

Y	JAN FEUSER ET AL: "Cell/adsorbent interactions in expanded bed adsorption of proteins" BIOSEPARATION, vol. 8, 1999, pages 99-109, XP002259859 abstract	4-26
A		1-3

Y	US 5 837 826 A (ROBICHAUD MICHAEL J ET AL) 17 November 1998 (1998-11-17) abstract	1,4-13, 15,17-26
A	column 1, line 49 -column 2, line 12 column 2, line 60 - line 64	2,3,14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2003	14 05 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mälin Söderström

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 03/00483

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 090 292 A (OTTO VEIT ET AL) 18 July 2000 (2000-07-18) abstract	1,4-13, 15,17-26
A	column 4, line 60 - line 64; figure 1	2,3,14
A	EP 0 955 312 A (SEIKAGAKU KOGYO CO LTD) 10 November 1999 (1999-11-10) summary, page 10, line 50 - page 11, line 53	1-26
P,A	WO 02/053251 A (LIHMES APS ;LIHME ALLAN OTTO FOG (DK)) 11 July 2002 (2002-07-11) abstract; figures 1-5	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK03/00483

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13, 14
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 1-26
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1. 3-15. 17-26.

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 13, 14

Claims 13 and 14 relates to methods of treatment of the human or animal body by surgery or by therapy or diagnostic methods practised on the human or animal body (PCT Rule 39.1(iv)), due to the sentence "obtaining blood from said mammal". Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds or compositions.

Continuation of Box I.2

Claims Nos.: 1-26

Claim 16 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. Claim 16 refers to steps (a), (b), and (c) in claims 12-15, which does not comprise these steps. It is not clear from claim 16 or the description why a substance is injected in the blood system or which substance is injected.

Following separate inventions have been identified:

1. Claims 1, 3-15, 17-26. An extracorporeal adsorption method for removing harmful substances using a column with adsorption medium in form of particles forming a fluidised bed. The particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria and/or Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.

2. Claims 2, 3, 5-12, 19-25. An extracorporeal adsorption method for removing harmful substances using a column with adsorption medium in form of particles. The particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria and/or Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.

D1 represents the most relevant prior art. From D1 is known a method purifying blood by using a column having particles with affinity to endotoxin of gram-negative bacteria, e.g. lipopolysaccharide from E.coli, see fig 2, column 4, line 61, claims 1-11. Therefore the common special technical feature, the particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria, for the two inventions are known.

Invention 1 and 2 are therefore not linked with a common special technical feature. Hence the requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between invention 1 and 2. Thus, the invention lacks unity Ó posteriori.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1, 3-15, 17-26

Claims 1, 3-15, 17-26. An extracorporeal adsorption method for removing harmful substances using a column with adsorption medium in form of particles forming a fluidised bed. The particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria and/or Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.

2. Claims: 2, 3, 5-12, 19-25

Claims 2, 3, 5-12, 19-25. An extracorporeal adsorption method for removing harmful substances using a column with adsorption medium in form of particles. The particles carry an affinity specific molecule having affinity for the LPS portion of Gram-negative bacteria and/or Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/DK 03/00483

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 1057529	A	06-12-2000	AU	1689900 A	12-07-2000
			EP	1057529 A1	06-12-2000
			US	6461517 B1	08-10-2002
			WO	0037172 A1	29-06-2000
			JP	2000237585 A	05-09-2000
			JP	2000237586 A	05-09-2000
US 5837826	A	17-11-1998	US	6036861 A	14-03-2000
US 6090292	A	18-07-2000	DE	19705366 A1	13-08-1998
			AT	200231 T	15-04-2001
			DE	59703294 D1	10-05-2001
			EP	0858831 A1	19-08-1998
			ES	2156331 T3	16-06-2001
			JP	11009688 A	19-01-1999
EP 0955312	A	10-11-1999	JP	11335396 A	07-12-1999
			CA	2264258 A1	25-09-1999
			EP	0955312 A2	10-11-1999
			US	6040291 A	21-03-2000
WO 02053251	A	11-07-2002	WO	02053251 A1	11-07-2002
			EP	1345666 A1	24-09-2003